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BIOLOGY OF THE PARASITES AND HYPERPARASITES OF APHIDS

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BY

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BIOLOGY OF THE PARASITES AND HYPERPARASITES OF APHIDS.*

HERBERT SPENCER

A biological study of the parasites of aphids was begun early in the summer of 1920, after many observations during an outbreak of *Aphis gossypii* Glov. on cotton in North Carolina had demonstrated the economic importance of the parasites and predators in natural control of the pest, and had aroused great interest by their efficiency. In the course of the work, similar parasites of other aphids were encountered, and hyperparasites were found closely associated. A general investigation of these insects and their interrelations followed. Opportunity for study of the Aphid parasite Complex in Ohio was afforded in 1921, and the project was continued there until July, 1922.

In this study assistance has been given freely by many, and grateful appreciation is expressed for this help, especially to Doctor Herbert Osborn, Research Professor of Entomology, Doctor Raymond C. Osburn, Professor of Zoology and Entomology, and Doctor Clarence H. Kennedy, Assistant Professor of Zoology and Entomology of the Ohio State University, and also Doctor Zeno P. Metcalf, Professor of Zoology and Entomology of the North Carolina State College.

*Contribution No. 88, from the Department of Zoology and Entomology of the Ohio State University.

HISTORICAL.

The earliest biological work with parasites of aphids was published in 1899 by Seurat. This writer studied the post-embryonic development of *Doryctes gallicus* Rhein., a Braconid parasite of certain Lepidoptera and compared the related species *Apanteles glomeratus* Linn. and *Aphidius fabarum* Marshall, the latter a parasite upon *Aphis rumicis* Linn. in France. The references to *Aphidius* are scattered through the paper, but when sought out, form a quite complete biological discussion of the development and structural modifications of the parasite itself.

Timberlake (1900) observed and described the early stages of *Praon simulans* Prov. and *Aphidius rosae* Hal., figured the first stage larva of the former, noted "inhibition" of extra larvae in cases of superparasitism, and pathological changes in what he considered the aphid fat bodies.

Ainslie (1909) discussed briefly the manner of attachment of parasitized aphids and considered that they are held to the leaves by "death grip" of the tarsal claws, or by a glutinous fluid forced through the pores of the aphid. In addition the larva afterwards rends the ventrum of the aphid and spins with its mouth a cocoon, attaching this also to the leaf through the opening, according to this author. He also recognized that aphids parasitized by *Aphelinus* are not split ventrally when attached.

Webster (1909) in a bulletin on the Spring Grain Aphis, *Toxoptera graminum* Rond. discussed control by *Lysiphlebus tritici* Ashm. (*Lysiphlebus testaceipes* Cress.) and figured the adult and egg of the parasite and also parasitized aphids. Experimental distribution of the parasite for control gave negative results. In certain parthenogenetic experiments a few females were obtained among many males in the progeny from unfertilized females.

Gahan (1911) in his systematic paper, included notes on habits, life history and hosts for each species of primary parasite of the subfamily Aphidiinae.

Webster and Phillips (1912) in a later bulletin on *Toxoptera graminum* Rond. discussed in detail the biology of *Lysiphlebus testaceipes* Cress., and of several other parasites and hyperparasites associated with these insects. This is our most comprehensive biological paper on aphid parasites.

Ainslie (1917) reported the peculiar formation of the cocoon in *Praon*, which spins underneath the skin of the parasitized aphid rather than inside the skin, as do the other parasites.

Haviland (1920) investigated the bionomics and development of *Lygocerus testaceimanus* Keiff, and *Lygocerus cameroni* Keiff., two European Proctotrypid hyperparasites of aphids through the *Aphidiinae*, and compared the stages of other Hymenoptera. The same author (1921) in an interesting paper on the post-embryonic development of three European Cynipid hyperparasites of aphids through *Aphidius* summarized briefly in a paragraph what is known about the relations between the primary parasites and aphids, and the tissue changes involved. The life history and development of the hyperparasites, *Charips* spp., were treated admirably, and comparisons were made with the development of other parasites.

Hartley (1922) treated in detail the life history and biology of *Aphelinus semiflavus* How. and certain relations between larvae of this species and larvae of Aphidius in the same aphid.

Wheeler (1923) discussed fully previous papers on the larval stages of the *Chalcidoidea*, *Proctotrypoidea*, and *Ichneumonoidea*, defined the larval types of parasitic Hymenoptera, and treated of the life histories of certain species of *Aphidiinae*, with special reference to the larval stages.

APPARATUS AND METHODS.

The insects used in this project were reared for the most part in home-made cages, each of which consisted of two six inch wooden embroidery hoops, over which was sewed a cylinder of fine-mesh cheesecloth, stretched by two thin strips of wood spacing the hoops; a square of glass covered the top and permitted observation of the plants and insects inside; the bottom of the cage was made of two glass plates which rested on the top of the pot, and which were suitably notched to fit closely around the base of the stem of the potted plant.

Aphids were grown in thse cages until quite numerous and then the adult parasites were admitted. A few aphids were removed from time to time for dissection, and at regular intervals numbers were fixed in Bouin's Picro-Formol or Brasil's Fluid for serial sectioning. Most of the insects were cut with

a microtome setting of ten micra, though some sections were made five micra thick. Delafield's Hematoxylin and Eosin, or Heidenhain's Iron-alum-hematoxylin stains were used, and the resulting slides demonstrated clearly the internal relations between larval parasites and hosts and also other points not clear from the gross dissections. The hyperparasites were handled in much the same way, or in shell vials where these were found to be suitable.

Many collections and observations were undertaken in the field, and every effort was made to understand the interrelations of the factors and species under normal outdoor conditions.

THE APHID COMPLEX.

PHYSICAL FACTORS IN NATURAL CONTROL.

From field observations both in North Carolina and in Ohio it seems that the factors concerned in natural control of aphids fall into two general classes, physical and biological. Under the first of these should be placed moisture and temperature. Moisture in the form of humidity probably exerts little direct influence on aphid abundance, but indirectly by working through certain of the biological factors to be discussed later, has considerable bearing on the problem; in the form of rain there is direct connection, as rain washes many aphids from plants and beats them into the mud where they die. Temperature is probably a more important factor. It is often observed that outbreaks of aphids seldom persist long after hot summer weather has set in. In order to throw some light upon the effect of various temperatures on aphids, a series of the cages was set up, in a chamber where the temperatures could be varied at will. It was found that *Myzus persicae* Sulz. living on dock (*Rumex* sp) and on potato plants behaved normally up to 30°C., but at 33°C. there was considerable restless activity and the winged forms flew away. At 35°C. all of the aphids were running aimlessly about and at 40°C. they began dropping from the plant; a temperature of 44°C. for ten minutes removed over half of the aphids, and a plant subjected to 49°C. for an hour was wilted, subsequently shed most of its leaves but recovered, and was thereafter free from aphids.

Comparable temperatures may be attained on sunny days in the open, close to the soil where radiation adds to the already high temperatures. In addition moderately warm periods have an indirect effect on aphid abundance, in that they encourage activity by certain of the biological agencies of control; this indirect relation is without doubt more important than the direct, as its influence is felt far ahead of the time when the extreme conditions which would bring about any direct control would be present.

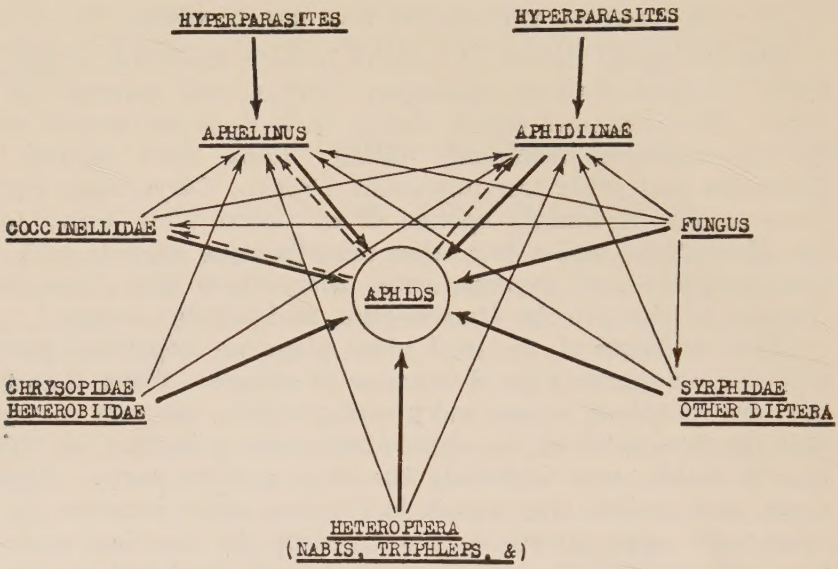
BIOLOGICAL FACTORS IN NATURAL CONTROL.

The biological factors of control include condition of food plant, fungous diseases, predatory insects, and parasitic insects. The predators count among their numbers several of the *Coccinellidae*, commonly called "Lady Bird Beetles," *Syrphidae* and other less important *Diptera*, *Chrysopidae* and *Hemerobiidae*, ordinarily spoken of as "Lace Wing Flies," a few *Heteroptera*, and a few other miscellaneous animals. The true parasites and hyperparasites with which this paper is concerned belong to the *Hymenoptera*, and are tiny wasps.

The condition of the food plant plays an important part in determining the rate of increase of aphids. Plant tissues that are succulent, sappy and growing rapidly are very favorable, as demonstrated by aphids habitually collecting on the tips of plants, and neglecting the more mature parts. Here again, wet periods play a part in favoring aphid increase, because such sappy growth is encouraged by the abundant moisture. Slow growing, well-seasoned tissues produced in dry periods are thus not as favorable to the aphids, possibly because of greater difficulty in obtaining food in proper quantity, so growth and reproduction on these are slower. For the same reason the ripening of plant tissues or the dying of the host is fatal to the aphids. This food plant relation is more or less basic for the whole Aphid Complex.

The relative efficiency of the various biological agencies of natural control varies with the time of year, and with differences in weather conditions. Early in the spring when the weather is still cool, the aphids usually get quite a start and increase in numbers very considerably. At this time of abundant precipitation, cloudiness alternating with sunshine, and moderately low temperatures, the fungous diseases of aphids

find favorable conditions and come into prominence. The Hymenopterous parasites are few in number and are not very active. Likewise, the Coccinellids, Syrphids, and Lace Wing Flies are too few to be of great economic benefit but they begin to multiply at this time. As the weather becomes warmer and more settled the fungous diseases lose first rank in control. The Lace Wings and *Diptera* seldom take prominence, but help materially the Lady Bird Beetles and the parasites. At this stage the number of aphids decreases rapidly and the



THE APHID COMPLEX BIOLOGICAL CONTROL.

Fig. 1. Heavy lines denote direct attack; light lines relation with aphid as intermediary (i. e., predator eats aphid containing larva of parasite); broken lines defense by aphid (i. e., gumming).

outbreak may subside; often after a short time the plant lice become so scarce that many of the parasites perish for lack of hosts. The predators in adult form may be able to migrate to other outbreaks of different species, as they are seldom as limited in the direction of suitable host species as are the parasites. At times there may be recurring outbreaks of the aphids of lesser extent, with subsequent reappearance and increase of parasites and predators, but usually these secondary waves are of little significance from an economic viewpoint. Often

after the primary outbreak a condition of balance will prevail for the remainder of the crop year, with a few aphids present, some parasites which are held down by hyperparasites, a few predators, and a small amount of fungus. There may be considerable competition among the insect agencies of control where aphids are few in number, and the activity of any predator or parasite may seriously restrict the opportunity of other parasites and predators, and in turn be limited by their work. Both food supply and opportunity for reproduction may be lacking when such conditions prevail. Then also, there may be direct destruction of beneficial insects by other beneficial insects, as where the young stages of the parasites inside of the bodies of aphids are eaten when predators devour such plant lice. The complex relations of the many factors concerned in biological control are shown graphically in Text Figure 1.

THE PARASITES OF APHIDS.

The Hymenoptera associated in the Aphid Complex are divided, because of their habits, into two groups: those parasitizing aphids directly, alluded to as the "Primary Parasites" or simply "Parasites," and the forms attacking stages of the primary parasites in aphids, spoken of as the "Secondary Parasites" or "Hyperparasites." To complicate relations still more, there is good evidence that certain of the hyperparasites at times attack other hyperparasites or even young stages of their own species. This is rarely accomplished in the Aphid Complex under natural conditions and probably has little economic bearing but nevertheless it is a relation of great interest. It is evident that the Primary Parasites are vastly beneficial in an economic sense, since they limit the reproduction and bring about the destruction of insect pests that are most troublesome. As the Hyperparasites in turn limit the activities of the Parasites, they are to be considered as harmful insects.

The Primary Parasites are assigned by systematic workers on the Hymenoptera to two diverse groups: (1) *Ichneumonoidea*, Family *Braconidae*, Subfamily *Aphidiinae;* (2) *Chalcidoidea*, Family *Aphelinidae*. There are many species of the first group, the *Aphidiinae*, found wide-spread thruout this coun-

try and the world; they are most active and prolific and are by far the most important of aphid parasites. When compared with their work, that accomplished by the second group, the *Aphelinidae*, is insignificant.

The Hyperparasites are scattered even more widely by systematists than are the Parasites. There are forms assigned to the *Chalcidoidea*, Families *Entedontidae* and *Pteromalidae;* some *Cynipoidea* belonging to the Family *Figitidae*, and a species of *Serphoidea* (*Proctotrypoidea*), family *Ceraphronidae*.

In reporting the results of this study a general discussion of the insects belonging to each group will be given; in the case of the *Aphidiinae* the general discussion of habits, development, and economic importance is based upon a thorough study of *Diaeretus rapae* Curt., *Lysiphlebus testaceipes* Cress., *Aphidius nigripes* Ashm., *Aphidius polygonaphis* Fitch, and *Praon simulans* Prov., and the data obtained thereby has been checked up as far as possible with the other species mentioned in the body of the paper. The other groups of parasites have been treated in a comparative manner also where this was possible.

THE APHIDIINAE.

HABITS OF THE ADULTS.

The life activities of the adult parasites are very interesting. Just after emerging from its cocoon in the dried aphid skin the parasite is soft and rather wet, but it requires only about five minutes for the wings to expand and dry and the body to become compact. During this period the movements of the insect are rather uncertain and poorly coordinated but it is surprising how soon the adult becomes fully mature. The adults are most active on bright sunshiny days when the temperature is between 20°C. and 30°C. At lower temperatures the parasites remain on the under sides of the leaves, more or less inactive. They are extremely sensitive to variations of humidity; excessive dryness will kill both parasites and aphids quickly, as has been demonstrated many times in cages and breeding vials. There is little activity among them on dark or rainy days or at night when they rest quietly on the lower leaf surfaces.

In the field the problem of obtaining food presents no difficulty to these insects. They have only to lick up the honey dew excreted by the aphids. In this respect they are messmates of a host of other Hymenoptera such as ants and many kinds of wasps. However the parasites do not "milk" the aphids and eat the honey dew direct from them, as some insects are reputed to do, but lap it up from the top of lower leaves which often become varnished over with the material dropped from above. In the cages and vials used in the breeding work, a mixture of clover honey and water was used for feeding, and all of the parasites lapped this up greedily until gorged.

Wintering: The method of wintering of the Aphidiinae parasites depends more upon the resistance of the plants on which the aphids multiply than upon the severity of the cold. The adult parasites are able to withstand temperatures that are surprisingly low. In experiments, adult *Diaeretus rapae* have been kept out-doors with no protection except a small glass vial in which they were confined, for periods of over thirty days, alive and well, but of course inactive, with temperatures ranging from -12°C. to 10°C. When these parasites were brought indoors, within fifteen minutes they were as active as in the summer. It was found to be impossible to carry any of these through the entire winter, however, as they became emaciated after about a month and died from starvation. The pupae of the parasites are even more resistant and serve to bridge over the winter, at least in the species mentioned. For example, on February 4, 1922, examination was made of the plants in a cabbage patch at Columbus, Ohio. The field had been cut over several times in the fall and most of the heads sold, but here and there were left poor plants that had grown somewhat, but at the time mentioned were badly injured by cold and half covered with snow and sleet. A half dozen plants revealed one live and thirty-two dead parasitized *Aphis pseudobrassicae*, which were brought into the laboratory. In five days six perfectly healthy *Diaeretus rapae* had emerged from those parasitized. They had been subjected to all sorts of weather in the winter, and it is surprising that so many were alive. Cold merely seemed to slow up their development. Undoubtedly the natural refrigeration of the pupae in winter serves to tide over this species until spring.

Aphids, if they are able to obtain food, can reproduce in sheltered locations even in very cold weather, but if the hosts die at first frost as do tomatoes or melons, the aphids upon them succumb to starvation and the parasites cannot survive; or if the plants ripen in hot weather as do the grains, the aphids may be destroyed just as thoroly by lack of food and the parasites find no hosts upon which to work. In such cases the parasites do not remain in the locality all year around. On the other hand, in places where growing conditions are more favorable and where plants do not die from frost or ripen too soon, the parasites are able to continue their reproduction and migrate in waves when conditions are suitable again. This was the case with *Lysiphlebus testaceipes* in 1921. In the fall the first frosts kill the melon, squash, or other host plants long before settled cold weather, and the remaining pupae transform into adults which finally perish. No viable pupae remain to be refrigerated over the winter as with *Diaeretus rapae* because settled cold weather does not follow soon enough after the first killing frost. In regions of the South, such as Florida or Texas, melons or cotton or other host plants may not be killed out, and may support the aphids and the parasites all winter. Then in the spring there is a wave of aphid migration northward, and a subsequent wave of parasites. For example, in 1921 *Lysiphlebus* reached Raleigh, N. C., on June 10, but did not appear at Columbus, Ohio, until September 6; *Aphidius nigripes* reached Raleigh on May 7 and disappeared on June 1, but reached Columbus early in June and disappeared by July 15.

There is one other possibility of wintering in the North. Greenhouses often become infested with aphids and it is possible that parasites would survive under the protection of these structures. Such a possibility is a remote one, although there is good evidence that in the winter of 1921–1922 *Aphidius phorodontis* came through in this manner in a conservatory at the Ohio State University upon aphids of tomato and other plants.

Mating: The females and males are ready for mating as soon as they have dried out after emergence. To sense the presence of the female the male has to be within a short distance of her, seven to ten millimeters being the limit. The female when approached usually tries to run away and the

male pursues and climbs on the back of the female, which seems to have a quieting effect upon the latter. Then the male crawls backward, curves the end of the abdomen over and under the tip of the abdomen of the female, and copulation ensues. This lasts from a few seconds to a minute and is usually terminated by the female breaking away and crawling off. Often, if many males are present, extra males will mount a mating pair and endeavor to copulate also; sometimes these will persist in their efforts to mate with the successful male long after the female has left. Several instances have been observed where a female was carrying about three males, one above the other.

Oviposition: Oviposition may occur with or without previous mating and fertilization of the female, and the response of a female to the presence of a suitable aphid is equally vigorous, whether virgin or mated. The act of oviposition has been described and illustrated by Webster (1909) and many other authors have copied or adapted his illustrations. Briefly, the process is as follows: The parasite comes up to an aphid, arches her antennae above it and taps it a time or two with each antenna; then if apparently satisfied, she curves her abdomen under her head and thorax, between the braced legs, and still facing the aphid, elongates the abdomen until the tip comes in contact with the body of the aphid; then there is a quick forceful stab and oviposition has been accomplished. Usually only one egg is placed in an aphid but where there are few aphids, as in certain of the oviposition experiments carried on in glass vials, or a superabundance of parasites, an aphid may be "stung" several times. The egg may be placed in any part of the body of the victim, even in the head. The aphids seem to suffer little or no inconvenience from the oviposition; a drop of exudate appears at the point of puncture but this soon hardens and no observable derangement is produced, except where the aphid has been oviposited in many times, when a slight sluggishness is manifested. For oviposition the *Aphidius* parasite ordinarily selects a half grown aphid, but when conditions are such that egg laying is vigorous, as on quiet, hot, sunny days, the parasite will attack aphids of any stage and will lay egg after egg in one individual if the supply of aphids is limited.

The process of oviposition is not entirely without danger to the parasite. If any part of the insect comes into contact with the cornicles of the aphid, it becomes "gummed" with the gluey secretion produced by the plant louse. This secretion hardens on the parasite and renders it temporarily "*hors-de-combat.*" The parasite will then spend half an hour or so scraping at the affected parts in an effort to remove the substance. Such "gumming" often produces permanent impairment of the functions of the tiny wasp, and may hasten death by several days.

THE EGG.

The mature ovarian eggs of these *Aphidiinae* are elongate oval structures with one or both of the ends tapering more or less into blunt projections. When completely developed in the ovary the measurements average, with *Diaeretus rapae*, 50 by 20 micra. In oviposition these are placed by the parasite in the adipose tissue of the aphid, usually with part of the egg projecting a short distance into the body cavity. Almost at once the egg absorbs liquid from the aphid by osmosis, and increases enormously in size, becoming lemon shape and as large as 130 by 60 micra. Plate VII includes drawings of ovarian eggs, Fig. 1, and eggs after oviposition and absorption of fluid from the aphid, Fig. 2.

EMBRYOLOGY.

The early stages in the development of species of *Aphidiinae* are exceedingly difficult to follow, for several reasons: the embryos are very small, their cells are densely packed together, the tissues are poorly defined, there is little or no yolk material, and it seems that many of the usual processes of embryonic development are either modified greatly or eliminated entirely, being bridged over by these parasites.

The youngest embryo obtained was a stage of *Diaeretus rapae* of approximately ten hours development. This lay in the adipose tissue of the aphid completely enveloped, and measured 30 by 50 micra. The chorion had disappeared except for a small sector originally around one of the projecting ends of the egg. The embryo itself contained many nuclei, arranged radially from the periphery toward the center, two or three deep. Between adjacent radii were noticeable divis-

ion lines reaching nearly to the clear center area. The most notable thing about this embryo is the light thrown by it upon the formation of the serosa, the only membrane formed by the embryo in these species. This embryo is shown in section as Figure 3.

The Serosa: At two or more points on the periphery of the middle section on the slide can be seen round cells, exactly like the cells in the embryo, but pushed outside of the outer layer. Adjacent to these are similar external cells partly flattened out and joining each other with their thin cytoplasm to form the membrane. Cells in all stages of transformation from the spherical ones just extruded to the typical flattened serosal elements are shown by this section. Thus the serosa is formed by delamination or extrusion of cells from the ectoderm of the embryo early in embryonic development. These flatten, grow both in amount of cytoplasm and in nuclear material, become polyhedral finally, and spindle-shaped in cross-section. The nuclei become oval in outline, considerably flattened, many times the size of the embryonic nuclei, and more open in construction as the chromatin masses are well separated. The cytoplasm is finely and evenly granular and is inclined to take the hematoxylin stain rather than the eosin. Stages in the formation and the later development of the serosa are shown in Figures 15, 16, 17 and 18.

Embryos of 24 to 48 Hours: Because of the methods of taking aphids for sectioning at regular daily intervals, the next stage in development, the embryo of twenty-four hours, appears many times in the slides of all species of *Aphidiinae* studied. In this there is considerable increase in size, in number of cells, and the serosa is completely formed and has grown to the point of fitting rather loosely around the embryo. The radial divisions between groups of cells are quite distinct, and the central space which is the segmentation cavity still persists. This embryo has a wonderful similarity to the "morula" stage so much stressed in some courses in Vertebrate Embryology. It shows little advance over the earlier embryo. Figure 4 is a section of an embryo of this stage, in its membrane.

During the second day the embryo elongates considerably in the future anterior-posterior direction, by cell multiplication; the segmentation cavity elongates likewise, but keeps the same diameter. The head region grows faster than the other

parts and from its greater diameter can be recognized. However, no tissue differentiation can be made out in any of these embryos, except that the cells of the outer layer appear very slightly smaller than those in the interior. In this period the rapid growth is seldom uniform throughout the body of the larva, and this unequal development brings about peculiar twistings so that the embryos appear for all the world as if they were "writhing in agony." The serosa is still present, having enlarged somewhat in the interval. This stage and the twenty-four hour and many intermediates were found in slides of *Diaeretus rapae*, *Praon simulans*, *Aphidius nigripes*, and *Aphidius polygonaphis*. It is delineated in Figure 5. A few hours more and the young larva untwists and takes a position in its membrane so that the head and posterior ends are approximated, and the body is looped in a quite regular curve; this is accomplished by an equalization of growth, not by the action of muscles. From this point onward development seems more rapid.

Embryos of 48 to 72 Hours: During the third day the size of the head increases enormously, external segmentation of the body occurs, and tissue differentiation is initiated. The serosa has become quite roomy and the abdomen of the embryo in growing uncoils more and more. In the early stages of the third day the nervous system mesenteron, proctodeum, the two Malpighian tubules, and the two salivary glands can be recognized as distinct structures, having become less densely packed together and separated by spaces connecting with the segmentation cavity. At this time the cells of all these organs are alike as to size, appearance, and staining capacity, and have not reached the point of specialization, appearing like those in the earliest embryo found. However, this specialization occurs by the end of the third day and then and thereafter the organs change little except in size, up to the last molt. Figures 6 and 7 represent embryos of the third day, reconstructed from serial sections.

Hatching: Some time during the third day the larva frees itself from the serosa and comes to lie in the body cavity of the aphid. It is possible that the membrane is torn asunder by the larva growing and becoming straighter, but it is more probable that the larva, beginning at the time of tissue differentiation, secretes a cytolytic enzyme that aids in breaking up

the serosa. Possibly there is a combination of these two processes. Certain phenomena to be discussed later lend strength to the enzyme hypothesis. It is a fact that hatching is not the result of violent muscular movements, since the muscles are just starting to develop, and furthermore the aphid tissues are never disturbed in the least, as would occur with activity of the larva of such a nature.

THE FIRST LARVAL STAGE

The larva of the first stage (Figure 8) is elongate and uncurved, or but slightly curved as it grows older, and is composed of a large head and thirteen body segments that taper gradually to the posterior end, which ends in an elongate "tail" in all species of *Aphidiinae* studied. In *Praon* this tail is double, or rather bifid. It has been suggested that this structure functions in aiding the larva to move about inside the aphid, or to prod about into the tissues of the latter to hasten disintegration and release food for the parasite. Both views are erroneous, for up to the time of the last larval instar there is no indication either in the larva itself or in the tissues of the aphid, that there is any voluutary movement of the larva, and there is certainly no mechanical change in the aphid tissues to substantiate the prodding theory. In the first larval stage, as in the embryo, absorption, respiration, and excretion are evidently carried on by the cells of the ectoderm of the parasite, by the process of osmosis. The food is carried to the larva in the blood of the aphid, and the oxygen likewise, and wastes are carried away by the same medium. The larval parasite develops in precisely the same manner as do the embryos of the aphid, and so there is little need for moving or foraging for its food.

The Fate of the Serosa: At the time of hatching the serosa is broken up into pieces, sometimes as many as thirty being left in the body cavity of the aphid, each piece varying in size and in number of nuclei. The fragments of the serosa round off, and begin a strange process of vacuolization and growth, assuming forms as in Figures 15, 16, 17, and 18. The nuclei enlarge also, break down, fuse and finally result in a shrunken mass of chromatin. The cytoplasm becomes more and more vacuolated as the parasite larva develops, and the size of the degenerated mass increases steadily until the larva is ready

for the last molt. What happens then to these degenerate masses will be discussed later. They have been observed by Timberlake (1900) and many others but their origin has been universally misunderstood, as they have been referred to the aphid fat bodies for their origin, and the process by which they arise has been termed "pathological changes in the fat body." The fat body of the aphid is modified by parasitism but in an entirely different manner, as will be explained in the following pages.

Inhibition of Younger Larvae: It has been noted by the author mentioned above and by others that only one adult parasite ever emerges from a parasitized aphid, even tho sevdral eggs were laid therein at the same time or at different times. To account for this the theory was advanced that in some manner all but one of the larvae are inhibited or suppressed by the successful parasite. This much is true, as the serial sections of aphids that have been superparasitized in this way substantiate these facts, and in addition suggest a probable manner of accomplishment. Shortly after hatching, the first stage larva is seen to be growing vigorously and rapidly. But younger larvae in the same aphid soon lag behind with their growth checked; their bodies shrink, the contours and organs lose their definiteness, and the cytoplasm of the cells seems to be abstracted, leaving the nuclei very prominent by contrast, and closely packed together. One glance at such a larva is enough to satisfy one that extensive destructive changes have taken place, and that the process of inhibition is a bio-chemical rather than a mechanical one, for never are there any evidences of mechanical injury such as would be present if the older larva had eaten parts of the younger, or otherwise molested it. Suppressed and normal larvae of the type discussed are represented in Figures 23 and 24.

Effect of Parasitism upon Adipose Tissue: Almost as soon as the parasite larva breaks out of the serosa the neighboring adipose tissue becomes affected. The cell boundaries become more pronounced, the cytoplasm more and more vacuolated, the nucleus shrinks and instead of being rounded takes on a very irregular outline. It would seem that the substance of the adipose tissue is sapped by the developing parasite and the cells are left in a starving condition. As the larval

parasite develops, the sphere of affected adipose cells correspondingly increases, until all of the tissue in the aphid is involved. From all indications in the serial sections the associated oenocyte cells are not affected in the least. A similar change in the adipose cells may be observed in old aphids that are producing a number of large embryos, but seldom is as much effect seen as is invariably present with parasitization.

The manner in which this destructive effect is produced is not entirely clear. It is certain that the young larva does not directly eat the tissue, since the serial sections show no evidence of the process, and the mesenteron of the parasite is absolutely empty of solid food and even of yolk, which we do not find in the embryo at any stage. The food is absorbed by the larva from the body fluids of the aphid, in a manner entirely comparable to that by which the aphid embryos get their own food. Seemingly the larval parasite has need of much more food for its growth than has a group of aphid embryos, if we judge on the basis of the extent of tissue involved. Undoubtedly adipose cells function in insects as storehouses of food, and serve to keep the available supply of this in the body fluids at a condition of balance. That is, if an insect must exert itself extraordinarily in travelling, or needs much food for transformation or the process of reproduction, that is supplied by the blood, and the blood is replenished from the adipose cells. When the period of stress is over and the insect feeds again, the soluble food in the blood becomes more concentrated and the adipose tissue builds up again from this excess. It is very evident that a parasite larva utilizes a vast supply of food at the expense of the aphid, to the point where all the adipose cells are starved. Figures 19 and 20 illustrate the effect of parasitism upon adipose tissue of aphids.

Effect of Parasitism upon Reproductive Tissues: The most remarkable changes in the aphid due to parasitism are to be seen in the tissues of the reproductive system. The embryos are especially modified; in whatever stage they may be at the time of hatching of the parasite they are arrested in their development, and as the larva of the parasite grows, large vacuoles and spaces appear in the embryonic tissues, the cytoplasm of the individual cells becomes thin as evidenced by difference in staining capacity, and the embryos finally degenerate into vacuolated masses. Strangely enough the

pseudova before the initiation of division of cells to form embryos are not affected until the young and old embryos have been rendered entirely unrecognizable. Camera lucida drawings of aphid embryos in two stages of degeneration from parasitization are shown in Figures 21 and 22.

Like the pseudova, the ova which develop into winter eggs in some aphids are arrested in development by the larval parasite, but show only slight change in structure. In some cases a thinning out of the yolk material is to be seen, but nothing more. From an economic point of view, the fact that parasitization brings about a cessation of reproduction of the aphid on the third day or thereabouts is an important consideration, since aphids may produce several young per day, and these are suppressed a week before the aphid itself succumbs to the parasite. In reckoning the economic value of these parasites in comparison with other control factors it would be very unfair not to give this point due weight.

Effect of Parasitism on Other Aphid Tissues: As far as could be observed parasitism causes no change in the muscular or nervous tissues. The digestive system in some cases seems to escape likewise, but at times there is to be seen in the serial sections a little of the same dissolving effect. It is of great advantage to the parasite larva that the nervous, muscular, and digestive systems of the aphid should remain intact until late in the process of parasitization. The last system furnishes food during all this time for the parasite, and the untouched muscles and nerves of the aphid mean that the latter stays on the plant and feeds, and does not fall off, starve, dry up, and die, leaving the unfortunate half developed larva inside to perish also.

MODE OF PRODUCTION OF ABNORMALITIES.

The means by which the larval parasite is able to produce such profound effects upon the cells of its own serosa, the younger larvae of its own species in the same aphid, the adipose tissue of the aphid, the embryos and pseudova of the latter, is worthy of careful consideration. It is clear that none of these can be mechanical effects, because the "tail" of the larva does not lacerate the tissue and indeed the larva does not move at all in the aphid in this stage. There is more involved than a mere starvation, since certain of the tissues concerned

show growth and peculiar hypertrophy. The theory of the writer is that the first larva arriving at the hatching stage molts and then begins to secrete a cytolytic or digestive enzyme, which frees it from its serosa, brings about the degeneration of the younger larvae, abstracts food from the aphid fat body, saps the developing aphid embryos and pseudova, in short, brings about the many cytolytic changes observed in connection with parasitism. The enzyme seems especially active upon growing and unstable tissues and affects only slightly the more stable parts such as digestive system, the nerves and the muscles of the aphid. The larva secreting this enzyme probably protects its own tissues by elaborating anti-ferments in the cells, as the cells of the vertebrate stomach are supposed to produce against the digestive ferments, which would otherwise digest the stomach itself.

THE SECOND STAGE LARVA

It is extremely difficult with species of *Aphidiinae* to determine with accuracy just when and where the molts occur in the process of development, since the stages are all internal and none are heavily chitinized, so the delicate shed exuviae are almost impossible to see even in the stained slides. For this reason differences in size, posture, development, body shape and internal organization have all been resorted to in order to determine with some accuracy the various instars. The second stage larva, Figure 9, is found ordinarily during the fifth day of development, although this time varies considerably for the different species, and even for larvae of the same species, for larvae in large aphids with an abundance of food develop more quickly than those under more adverse circumstances, and variations in temperature also seem to influence the rate of growth a great deal. This second stage larva is distinguished from the first stage by its larger size and by the greater curvature assumed by the body; also the anal process or "tail" is larger but more blunt than in the preceding larva. Nutrition and respiration, as in the previous stages, are entirely cutaneous, as there is no connection yet between the mesenteron and the outside, and the tracheæ have not reached a point of development that would permit functioning. Part of the excretion may be accomplished by the Malpighian tubules, as these seem mechanically capable of discharging their function at this stage.

THE THIRD STAGE LARVA

The distinguishing features of the third larval stage, Figure 10, which is found in some species about the sixth day, is the inflation of the abdomen by growth, so that the tail is no longer prominent. Internally the mesenteron has become much more capacious, but remains empty and shut off from the exterior. The salivary glands by their intense staining reactions reveal great activity, and two mandibles appear at the mouth, from which the stomodeum leads half way to the anterior wall of the mesenteron. Nutrition, respiration and excretion are still the same. The vacuolated masses derived from the serosa are now very large, surround the larva, and probably act as cushions in giving it mechanical support and protection. Until the end of this stage the aphid continues its vital processes in a more or less normal or subnormal fashion. Its reproduction was suppressed on the third day, and peculiar progressive internal changes started then, but in spite of this it appears to be normal enough that one could not definitely say from external evidences, whether or not it had been parasitized.

THE FOURTH STAGE LARVA

At a certain point in the development of the nearly grown larva there is a molt and a complete change of relations. The passive larva, fed by absorption and obtaining oxygen in the same way, becomes very active and commences to eat out the internal organs of the aphid with its well developed mandibles. When this begins the aphid moves no more, but grips the leaf in a last effort. The embryos or ova disappear first, then the digestive tract, the adipose tissue, the degenerated masses derived from the serosa of the parasite and the aphid embryos, the muscles of the thorax and abdomen, and the nervous system. The appetite of the larva, and its frantic efforts to satisfy this, are striking to behold through the semi-transparent aphid skin. In half a day or a day the aphid skin is empty, except for the larva and the parts in legs and head that could not be reached. During the eating-out process the plant louse turns a darker pearly color that is quite characteristic. Before the aphid skin begins to dry the larva chews away the entire area of ventral skin that is applied to the leaf surface.

In the larva other changes take place. The mesenteron previously small and empty, expands to twice and thrice its former diameter to accommodate the ingested food. The spiracles begin to function and the salivary glands become even more prominent and active, as evidenced by their more intense staining reactions. Before the aphid skin dries, the larva begins spinning its delicate silken cocoon inside this from the salivary glands, squirming and writhing the while with the result that the threads are quite evenly distributed. The skin of this stage is closely set with small tubercles, which probably grip the aphid skin during the squirming and thus assist the larva in moving about inside the shell. The silk adheres to the inside of the aphid skin and to the leaf in the spot where the skin has been devoured, and hardens quickly. Strand after strand is added in a hit-and-miss fashion until there is a uniform opaque layer on all sides. The space ventrally, where the silk is in contact with the leaf surface, is an added anchorage for the parasitized aphid and forms a final means of attachment, as the legs of the aphid soon dry, warp, and lose their hold. The fourth stage larva, Figure 11, now rests a day as if exhausted by its great activity, voids the undigested remains of its gluttonous meal as a meconium, Figure 12, and starts transforming into a pupa.

THE PUPA

The pupa, Figure 13, upon attaining complete development, transforms into the adult, Figure 14, inside of the cocoon. With the sharp mandibles the latter chews a neat circular hole thru the aphid skin, just large enough for it to crawl thru into the open. Sometimes the circle cut out is left attached by a small hinge and looks like a lid or cover but more often this is lost, either being cut loose by the parasite or being broken off in the process of emergence. There is left behind the dried aphid skin, and inside of this a string of about six to a dozen small black objects, very much like a miniature string of short sausage links, which are the remains of the dried meconium, Figure 12.

THE APHELINIDAE.

The majority of the species of the genus *Aphelinus* are parasitic upon scale insects, and only a few attack aphids. In the course of these investigations the blackened aphid-skins containing pupae of *Aphelinus semiflavus* Howard were encountered twice during 1921 at Columbus, Ohio, both times a single example in *Aphis pseudobrassicae* Davis upon kohl-rabi. No other species of the Family were obtained. This poor showing of *Aphelinus* is not difficult to explain: first, this parasite is not as vigorous in oviposition as the more active *Aphidiinae*, which destroy many more aphids; second, in respect to distribution, *Aphelinus* is a poor traveller, being disinclined or unable to use its wings for flight; the individual seldom moves quickly, except to hop like a flea when disturbed; lastly, a female will seldom attempt to oviposit in a moving aphid, or an old aphid, seemingly preferring the younger and weaker stages, and thus the reproduction of the old stem mothers is not curtailed as is often the case with those attacked by the Aphidiine parasites.

The biology and life history of *Aphelinus semiflavus* Howard have been studied recently by Hartley (1922) in considerable detail. This writer states that the winter is passed in the pupal stage, and the adults after emerging have been kept alive as long as 39 days, producing as many as fifteen eggs a day during part of this time, though the average is much less. Males are rare and parthenogenesis is the rule, females being produced without fertilization. Feeding is usually accomplished by the female sucking aphid juices thru ovipositor puncture holes. The eggs measure .21 by .05 mm., are oval and taper at each end. They hatch in three days and the larvae complete development in from five to nine days, feeding internally with their mandibles this while on the organs of the aphid. The author adds to the list of host aphids *Macrosiphum pisi* Kalt., *M. granarium* Kirby, *M. sanborni* Gill., and *Anuraphis viburnicola* Gill. Larvae of *Aphelinus* in aphids with larvae of *Aphidius* are arrested in growth and disintegrate; as reasons for this cannibalism, starvation, toxic substances, and lack of oxygen are suggested as possible causes and discussed. *Asaphes americana* Gir. and *Alloxysta* sp.

are mentioned as hyperparasites. Excellent figures of the adult in the act of oviposition, and of developmental stages are included.

The work done by the present writer in connection with *Aphelinus semiflavus* substantiates the observations of Hartley in every essential respect, and in addition throws light upon several minor points not brought out by that worker. Serial sections of aphids infested with larvae of both *Aphelinus* and *Aphidius* show that the former are arrested in development and caused to disintegrate not by starvation or lack of oxygen, which as Hartley says would surely affect all the larvae and not just the *Aphelinus*, and would not explain the fact that when two *Aphidius* larvae and one *Aphelinus* larva are in the same aphid, only one *Aphidius* grows and the other and the *Aphelinus* are suppressed; nor by cannibalism since the *Aphidius* larva does not use its mandibles until long after the effect has been produced, and the larvae are always uneaten in the serial sections; and not by toxic substances in the sense of poisons or the like, since such agents would hardly be innocuous to one *Aphidius* larva and destroy the other and the *Aphelinus*. The true state of affairs as revealed by the serial sections is this: one *Aphidius*, the older, appears vigorous and like all rapidly growing tissues takes a deep stain. The disintegrating larvae of both species show their cytoplasm very weakly stained as if nearly all of that substance had been abstracted. The nuclei are dark and show no change but appear by contrast very clear and distinct. As explained previously the older *Aphidius* larva upon reaching a certain stage in development evidently starts secreting a cytolytic enzyme which affects the tissues of the *Aphelinus* larva, younger *Aphidius* larvae, and aphid tissues. Thus the *Aphidius* eliminates competition by secreting a cytolysin which abstracts essential materials which it probably uses for its own growth, and not by means of a toxic substance in the sense of a poison.

Sections of aphids with *Aphelinus* larvae "in situ" are delineated as Figures 33 and 34.

THE HYPERPARASITES.

Lygocerus niger Howard.

Lygocerus niger Howard was secured in the field from parasitized *Macrosiphum rudbeckiae* and *Macrosiphum ambrosiae* on ragweed, *Ambrosia trifida* Linn. and Jerusalem artichoke, *Helianthus tuberosus* Linn. in September 1921, when it was found to be associated with *Aphidius polygonaphis*. Laboratory experiments with *M. rudbeckiae* of various ages, unparasitized and parasitized by *Aphidius polygonaphis*, showed that *Lygocerus* oviposits only in those parasitized lice from which the internal organs have been entirely eaten out; that is, *Lygocerus* is a hyperparasite and attacks *Aphidius polygonaphis* in the last larval and early pupal stages. Laboratory experiments indicate that *Lygocerus* will also parasitize similar stages of *Diaeretus rapae* and *Aphidius phorodontis*. In these three hosts seven generations have been reared in the laboratory up to January, 1922, from the original stock.

An interesting relation has come to light in connection with the laboratory breeding work. Several times upon dissecting stages of *Lygocerus* out of the aphid skins young pupae and full grown larvae of the species have been found which bore eggs of *Lygocerus* upon them. These eggs develop normally into adults, certainly a peculiar circumstance for the species to parasitize its own younger blood brothers and sisters. Thus *Lygocerus* is not only a Secondary Parasite but may be a Tertiary Parasite under these conditions. It is entirely possible that the Tertiary Parasite in turn could be parasitized in the same manner, and so on until so much of the original amount of food was used up in the process that the last parasite would not have left enough to complete development.

The adult *Lygocerus*, Figure 32, is an active nervous parasite, differing greatly in appearance from the other forms encountered. In ovipositing the female searches over the aphid community with her antennae, which are never still, until one of the rounded inflated aphid skins containing an *Aphidius* larva or pupa is found. Over and over this she crawls, investigating it thoroly with quick, continuous, alternating tapping of the two antennae. If apparently satisfactory, the female mounts the parasitized aphid, bends the tip of the

abdomen to touch it, and starts drilling in with the ovipositor. When fully exserted the ovipositor is unsheathed until its base at the middle ventral part of the abdomen is exposed, and the entire length of the apparatus is pushed into the aphid skin. Differing with *Aphidius* again, where oviposition is but the act of a second, *Lygocerus* consumes several minutes, seemingly exercising great deliberation and care in the process.

The egg of *Lygocerus niger*, Figures 25 and 26, when newly laid measures 0.25 by 0.10 mm. In one plane it is oval, with one end tapering more than the other; in the other it appears curved a little, being slightly concave at the place where it is applied to the body of its host. The surface is finely striated in the direction of the long axis and at each end there is a small teat-like projection. The egg is ordinarily attached somewhere near the middle of the body of its victim, well down toward the ventral side, in the curvature produced by the cramping together of the head and posterior end of the larva or pupa in the aphid skin. Usually only one egg is laid in a parasitized aphid, but two have been observed several times. In the latter case the extra egg is not placed adjacent to the one first laid but apart from it at some other point on the body of the host, several segments away. In the course of development one larva of the two gets the upper hand and consumes the other, so that only one adult issues. After the normal oviposition, incubation of the egg takes from a few hours less than a day to a full day. Some hours before hatching takes place the digestive tract and other organs of the developing larva can be seen thru the chorion.

In hatching the larva eats its way out through one end of the egg, and the chorion is left in place, much wrinkled. The first stage larva, Figure 27, is the same length as the egg, and tapers posteriorly; the mandibles are on the ventral side of the head and are slender, small, thorn-like structures, very sharply pointed. When exposed to the light by dissection, much activity is displayed by this larva, though it is probable that normally it remains attached near the middle of the host, sucking its juices through a small puncture made by the constantly moving jaws, seldom or never leaving place, as the skins of the many victims examined show only the one puncture spot. The first larval stage lasts about one day.

The second stage larva, Figure 28, is much larger, especially in head and abdominal region, which have grown faster than

the thorax. The habits are the same, and the duration of this instar is twenty-four hours, on the average.

The third stage larva, Figure 29, is fat and short, measuring as much as 1 by 0.5 mm. when fully fed. The host by this time is very much shrunken but the skin is intact except at the point of puncture. The food of *Lygocerus* is composed of the body liquids of the host, and the internal organs of the latter pulped by the mandibles. The eating process can be easily observed in specimens dissected out of their aphid skins. The mandibles work vigorously all the time and swallowing movements of a paristaltic nature carry the food into the midgut to add to the gluttonous store already there, which because of its yellow color is plainly visible by transmitted light. The third larval stage lasts one and one-half days on the average, but varies a half day each way in some cases.

The fourth stage larva, Figure 30, measures 1.4 by 0.7 mm. The body is tapering, has many short blunt spines or small tubercles on its dorsum and in rows down the sides of each segment, and there is a prominent dorsal projection in the form of a blunt cone on the posterior end of the abdomen. Haviland (1920) has found this in two related European species and suggests a locomotory function. Perhaps this is correct, and yet *Aphidius* larvae living under the same conditions in the same aphid skins are able to move about most actively when spinning cocoons, by the aid of small tubercles such as *Lygocerus* itself possesses, and without the caudal process. Indeed *Lygocerus* need not move about at all, as its cocoon has been spun for it by its victim, and its resting place is all ready for it. During this instar the remains of the host are usually consumed, skin and all; other larvae, seemingly less hungry, leave the host's skin, flattened and sucked dry of contents, and the meconium together as a scale in one end of the skin of the aphid. Just before pupation the *Lygocerus* voids its own meconium, Figures 31 "Mec," which takes the form of a sphere, differing greatly in appearance from the divided meconium of the Primary Parasite. The fourth instar lasts from one and one-half to two days.

There is an interesting kind of peristalsis in the midgut of nearly all larval stages. A broad constriction appears at the anterior end of this part of the digestive system and travels slowly backward to the posterior end, taking about four sec-

onds to reach this point. Then the direction is changed and the constriction moves forward to the anterior end at the same rate, then backward again and so on. The lumen of the constriction is evidently quite small since the particles of food swirl and squirt thru as it moves backward and forward, an unique method of mixing food and digestive juices, possibly resulting in rapid and efficient digestion and absorption.

The pupa, Figure 31, is yellow at first but grows gradually darker until it is black. It lies bent inside the aphid skin, with the ventral surfaces of head and abdomen somewhat approximated. The spherical meconium is found in the space between the abdomen and the head, among the appendages. The abdomen of the pupa, upon stimulation, moves from side to side and back several times much as do certain Lepidopterous pupae, except that the latter twist the abdomen around in a rotary manner. The pupal stage lasts from six to eight days.

A generation of this parasite can be produced in eleven days, and the average time is about twelve days, although a few straggle out to fourteen or sixteen days before emerging.

By dissecting out of the aphid skins just before they were ready to emerge, females were obtained that were unmistakeably virgin. These were placed singly in breeding tubes with numbers of aphids containing *Diaeretus rapae* in the last larval stage. Oviposition was seen to start at once. The progeny, averaging sixteen, were as numerous, as vigorous, and as large as for the mated individuals of the stock series of tubes, but no females were produced, every emerging *Lygocerus* being a male.

Asaphes americana Gir.

In the present work *Asaphes americana* Gir. was found associated with *Diaeretus rapae* Curt. at Raleigh, N. C., in February, 1921. The species also occurred as a hyperparasite on the same host at Columbus, Ohio, and was present until the fall of the year. It reached its greatest abundance in July and August when over fifty-five percent of the parasitized aphids on crucifers bred out this species. After October the numbers dwindled rapidly and during the winter none appeared tho many of the primary parasites were bred from field material. In addition during the past year *Asaphes* has been

bred from aphids parasitized by *Aphidius phorodontis* Ashm., *Aphidius polygonaphis* Fitch, and *Lysiphlebus testaceipes* Cress. Hartley (1922) reports it as a parasite on *Aphelinus semiflavus* How. in the greenhouse during late winter. It is evident that *Asaphes* can reproduce in a number of hosts and one is led to believe, from this list, that aphids parasitized by any of the primary parasites might be subject to attack.

Asaphes emerges from the inflated aphid skin thru an irregular circular hole that differs considerably from the larger, smoothly cut emergence hole of *Aphidius*. Mating occurs as soon as the female is dry, and oviposition as in *Lygocerus* may occur with or without fertilization. For oviposition the female of *Asaphes* hunts an aphid skin containing a late larva or early pupa of *Aphidius* or other primary host. Never is an unparasitized aphid selected or a parasitized one in which the larva has not eaten out the aphid and spun its cocoon. Thus beyond all doubt *Asaphes* is a secondary parasite. Aphid after aphid is investigated by *Asaphes* with her delicate antennae until such a one is found, and then after feeling all around the skin, the little wasp climbs up on the aphid, catches hold with all six legs, squats a little, points her antennae downward and starts drilling with the tip of the ovipositor. It takes all of five minutes constant work for the egg to be placed. The larva develops in an ectoparasitic manner, very much as does *Lygocerus niger*.

To determine the number of eggs laid by one female, and approximately the length of life, a series of experiments were carried on in the laboratory, in all, seventeen different females being studied. The table below gives the results for five females that are representative:

ASAPHES. LENGTH OF LIFE AND NUMBER OF PROGENY.

FEMALE.	DAYS LIVED.	PROGENY.	NOTES.
No. 8231	14	49	A virgin; progeny all males.
No. 9061	11	51	Six females, others of progeny all males.
No. 9062	23	76	A virgin; progeny all males.
No. 9091	14	90	A virgin; progeny all males.
No. 9221	7	5	Two of these females.
Average	14	54	

It will be seen from the above data that each female in her fourteen days of life can parasitize at least fifty-four *Aphidius*, and thus accomplish a great deal of harm by destroying these beneficial insects. The wonder is that any of the *Aphidius* are left with so much in favor of the hyperparasite. However in these experiments indoors a large percentage of males was produced and only a few females, even tho the parent had been fertilized. If this holds for field conditions, the danger from *Asaphes* is lessened. It is interesting to note that no females were among the progeny of virgins. Somehow or other, it seems to be the faculty of nearly every species of aphid parasite and hyperparasite to be able to reproduce parthenogenetically, and in every case but *Aphelinus* the virgin female begets males.

The males can easily be distinguished from females in *Asaphes*, because the former have a much less robust body, and antennae with spatulate tips, whereas the female is larger, possesses an ovipositor under the deeper abdomen, and has the tip of the antenna more conical and tapering. The antenna character is the most dependable, as the size varies with the size of the aphid host in which development takes place.

Pachyneuron apidivorum Ashmead.

Pachyneuron apidivorum Ashm. was bred from *Aphis rumicis* Linn. on September 14, 1921, from nasturtium plants at Columbus, Ohio. The mated females were given young of this aphid and of *Aphis pseudobrassicae* Davis for oviposition but paid no attention to them. However, when placed among aphids that had been previously parasitized by *Lysiphlebus testaceipes* Cress. and *Diaeretus rapae* Curt., whose larvae had just spun their cocoons, no time was lost by the *Pachyneuron* females, which began at once to lay their eggs. Oviposition and development was similar to the processes in *Asaphes*.

The length of time required for the development of egg into adult, from three generations reared during September and October, in *Aphis pseudobrassicae* parasitized by *Diaeretus rapae*, was from twelve to fifteen days, with the majority of the adults appearing on the thirteenth day after oviposition. The fecundity of the females was rather low, the greatest number of eggs laid by one individual being eleven. On ac-

count of this last fact, it is doubtful if *Pachyneuron* will ever play a very great part in the Aphid Complex, unless the habits of the species change greatly under the influence of different conditions that would be more nearly optimum for the parasite. Perhaps it is well that it cannot do more damage than it does.

Xystus brassicæ Ashmead.

In May, 1921, *Xystus brassicae* Ashm. was observed among *Aphis pseudobrassicae* Davis on cabbage plants at Raleigh, N. C., in association with *Asaphes americana* Gir. and *Diaeretus rapae* Curt. Several attempts were made to induce *Xystus* to oviposit directly in *Aphis pseudobrassicae* but without any success, in spite of the fact that many individuals were bred from parasitized aphids of this species from the field, over twenty-five percent of the infested aphids breeding out this species at this time. During the summer at Columbus, Ohio, *Xystus* was also obtained from a number of other aphids, being in each case associated with species of *Aphidiinae*. In late summer it was conceived that *Xystus* might be a hyperparasite on these aphids, developing only upon the developing primary parasites. To test this out two dozen *Xystus brassicae* were introduced into a breeding cage in which *Diaeretus rapae* had been rearing on *Aphis pseudobrassicae* for months. Oviposition in certain of the live aphids was observed at once. It was determined that only those aphids were selected which contained half-grown larvae of *Diaeretus*. Serial sections showed that the larval *Xystus*, (Figures 35, 36, and 37) develops in the body cavity of the larva of *Diaeretus* in a manner entirely similar to the development of the latter in the body of the aphid. Around the larva is a similar serosa, and feeding and respiration occur by osmosis at first, without destruction of the host larva, which is allowed to proceed past the point of cocoon spinning and to pupation before the hyperparasite larva induces destructive changes in the tissues of the primary larva in the same manner as the latter treats the aphid. From this point onward *Xystus* develops at the expense of its host and finally ends by eating the remnants of the latter. It then voids its meconium and pupates, but without spinning a cocoon, since the *Diaeretus* larva has already done this. The adult, Figure 40, eats its way out, leaving an irregular rounded hole, much different from the emergence hole of the *Aphidiinae*.

In the breeding cage mentioned the new generation of *Xystus* appeared in great numbers on November 21, just twenty-six days after the adults were introduced into the cage. The numbers of *Diaeretus* were cut down to about one-tenth, and in another generation disappeared. Then, having been too successful, the *Xystus* parasites perished also.

In the field during the summer of 1921, this parasite was watched with considerable interest and care. It was found most abundant in *Diaeretus rapae*, rather plentiful in *Aphidius polygonaphis* and *Lysiphlebus testaceipes*, and a few times in *Aphidius phorodontis*. A closely related species, *Xystus tritici* Fitch, so much like the species under discussion as to raise doubts about it really being a distinct species, was bred from *Aphidius nigripes* in *Macrosiphum granarium*. The relative abundance of *Xystus brassicae* and its hosts fluctuated considerably during the year of 1921, varying with each variation in number of aphids and parasites, but was always present in numbers great enough to seriously lessen the efficiency of the primary parasites. It was least abundant in the early spring and hot summer months, and most prolific in late spring and late fall thru the winter. The abundance of *Xystus* in pupal condition during the winter was surprising and of importance, as it settled the question of the method of wintering. From one set of twenty-five parasitized *Aphis pseudobrassicae* secured in February at Columbus, there were bred eight *Diaeretus rapae* and sixteen *Xystus brassicae*, a parasitization of two-thirds of the primary parasites. Other lots gave similar results.

INTERRELATIONS.

The relations between the species of hyperparasites, parasites, aphids and plants are complicated in many respects, and difficult to express in a connected manner, but a treatment of the Aphid Parasite Complex would be incomplete without a full discussion of this phase. So a recapitulation of the relations as found is presented in a graphic manner in Text Figure 2.

In this diagram are brought out several significant points. First, the number of important crops in the list of plants infested by aphids of this complex indicates somewhat the economic importance of these forms and their parasites, and justifies

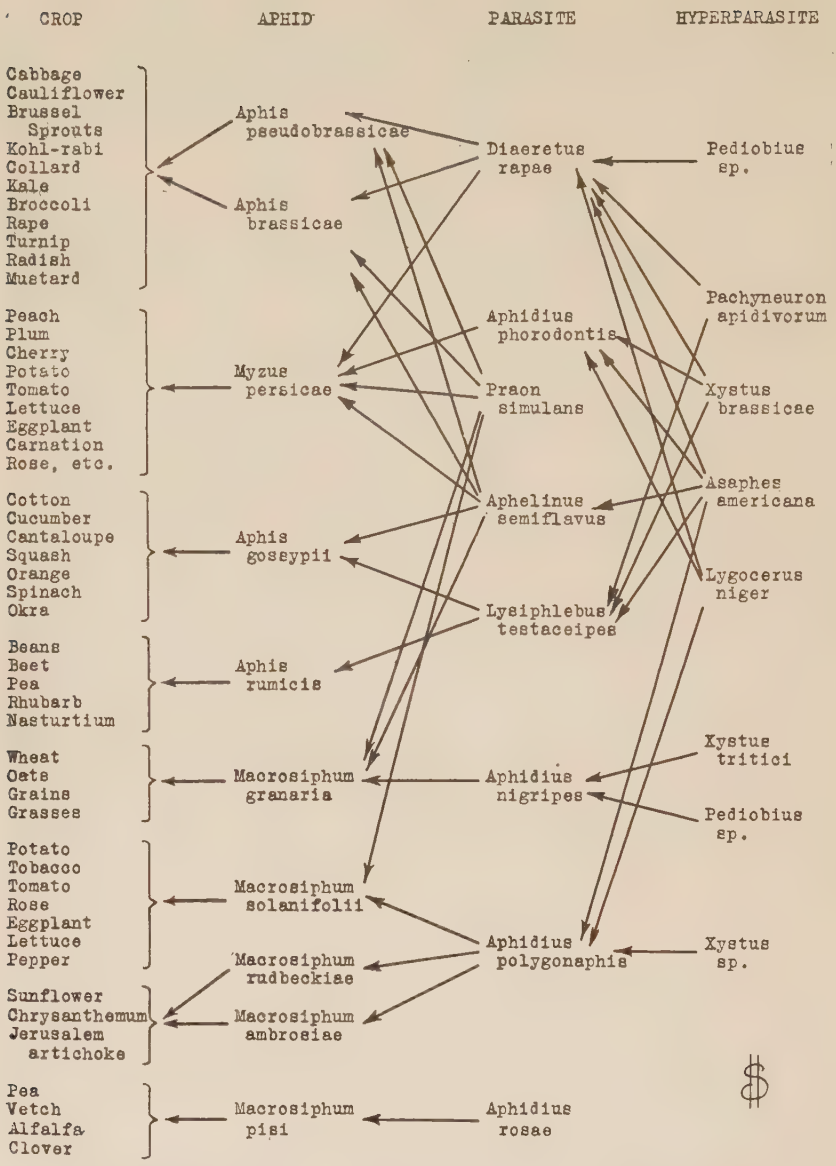


Fig. 2. The Aphid Parasite Complex. Relations between Crops, Aphids, Parasites and Hyperparasites.

a study of the biology of the latter since they are very beneficial. Second, the primary parasites are narrowly restricted to a few aphid hosts in most cases, which is a decided disadvantage from an economic standpoint; but this disadvantage is offset by the abundance of closely related parasite species, so that each one of the pests mentioned has at least one parasite to check its activities. Lastly, the hyperparasites are less restricted as to hosts than the others, perhaps a serious consideration from the economic viewpoint, yet not always so practically, since the life of a hyperparasite is very precarious at best, for if one of the factors upon which it depends drops out or changes very much, it means the end for the hyperparasite.

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EXPLANATION OF PLATES.

Plate VII.

Development of the *Aphidiinæ*, as illustrated by *Diaeretus rapæ* Curt.

Fig. 1. Ovarian egg. (× 380).
Fig. 2. Egg after oviposition. (× 380).
Fig. 3. Cross-section of ten hour embryo, from serial sections, showing formation of the serosa. (× 380).
Fig. 4. Cross-section of twenty-four hour embryo. (× 380).
Fig. 5. Embryo of forty-eight hours, reconstructed from serial sections. (×150).
Fig. 5a. Cross-section of this embryo. (× 350).
Fig. 6. Reconstruction of embryo of about fifty-five hours. (× 200).
Fig. 7. Embryo of the third day. (× 200).
Fig. 7a. Cross-section through head of this embryo. (× 200).
Fig. 8. First stage larva, reconstructed from sections. (× 50).
Fig. 9. Second stage larva, reconstructed. (× 50).
Fig. 10. Third stage larva, reconstructed. (× 50).
Fig. 11. Fourth stage larva. (× 50).
Fig. 12. The meconium voided by mature larva. (× 50).
Fig. 13. The pupa. (× 50).
Fig. 14. Adult *Diaeretus rapae*. (× 25).

Plate VIII.

The effects of Parasitism upon various tissues.

Fig. 15A. Surface view of portion of serosa, *Praon simulans*. (× 150).
Fig. 15B. Section through normal serosa, *Praon simulans*. (× 150).
Fig. 16A. Serosal masses after hatching of parasite larva of *Praon simulans* in *M. solaniifolii*. (× 150).
Figs. 16B, 16C, 16D. Masses from later stages, *Praon simulans*. (× 150).
Figs. 17A, 17B, 17C. Similar serosal masses from *Aphis gossypii* parasitized by *Lysiphlebus testaceipes*. (×150).
Figs. 18A, 18B, 18C, 18D. Transformation of similar serosal masses from *Aphis pseudobrassicæ* parasitized by *Diaeretus rapæ*. (×150).
Fig. 19. Normal adipose tissue from *Macrosiphum solanifolii*. (× 800).
Fig. 20. Adipose tissue from an aphid, *Macrosiphum solanifolii* parasitized by *Praon simulans*. (× 800).
Fig. 21. Normal embryo from *Aphis pseudobrassicæ*, sagittal section. (×125).
Fig. 22. Embryo from an *Aphis pseudobrassicæ* parasitized by *Diaeretus rapæ*. (× 125).
Fig. 23. Young larva of *Aphidius polygonaphis* Fitch, from an aphid containing an older larva of the same species. (× 75).
Fig. 24. The normal larva of *Aphidius polygonaphis* Fitch, from same aphid as Fig. 23, and responsible for the degenerate condition of that larva. Reconstructed from sections. (× 75).

Plate IX.

The development of the Hyperparasite *Lygocerus niger* Howard.

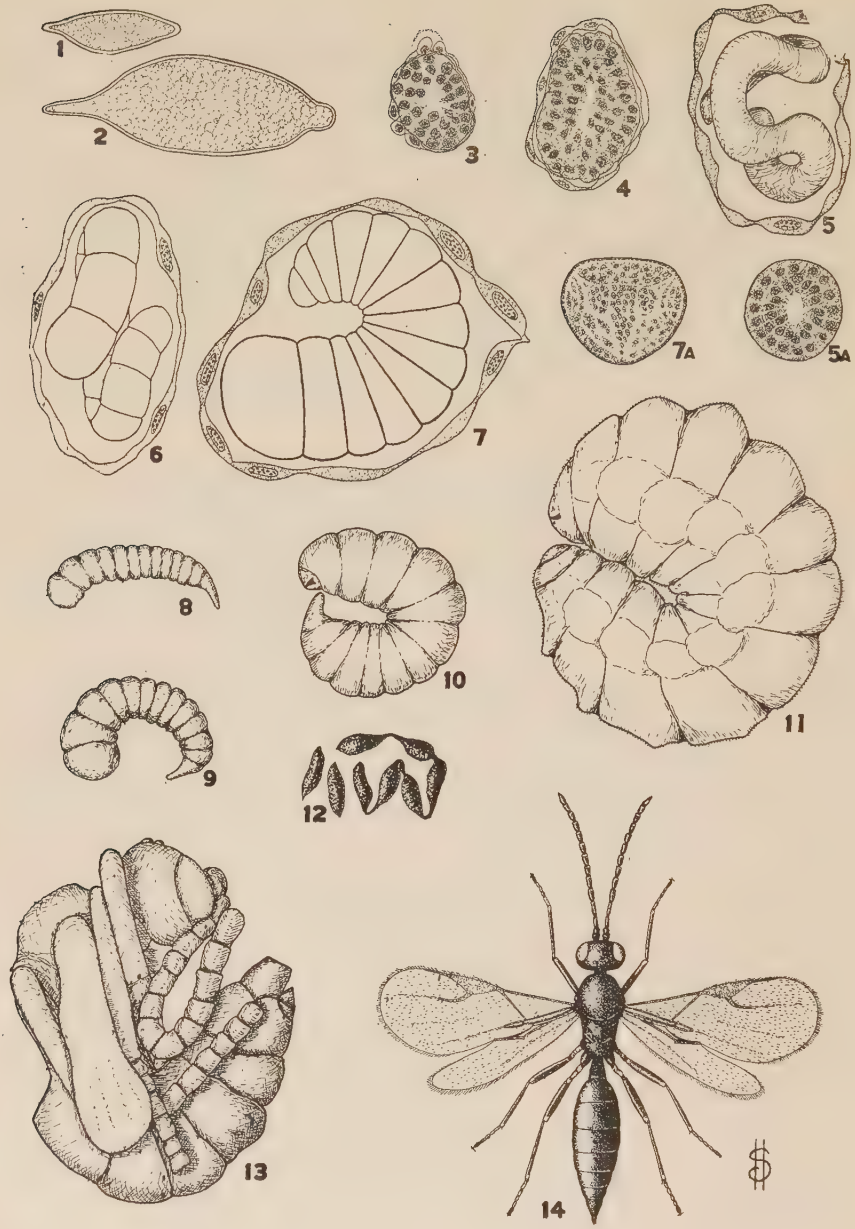
Fig. 25. Egg, dorsal view. (× 75).
Fig. 26. Egg, lateral view. (× 75).
Fig. 27. First stage larva. (× 75).
Fig. 28. Second stage larva (HYP) attacking and eating fourth stage larva of *Diaeretus rapæ* (PAR). (× 37).
Fig. 29. Third stage larva (HYP), attacking and eating pupa of *Diaeretus rapæ* (PAR). (× 37).
Fig. 30. Fourth stage larva (HYP), eating the remains of a pupa of *Diaeretus rapæ* (PAR). (× 37).
Fig. 31. The pupa, with meconium (MEC). (× 37).
Fig. 32. The adult female *Lygocerus niger*. (× 20).

(Figures 25 to 31 are from dissections of parasitized *Aphis pseudobrassicæ*).

Plate X.

The Parasite *Aphelinus semiflavus* Howard and the Hyperparasite *Xystus brassicæ* Ashmead.

Fig. 33. Cross-section of an *Aphis pseudobrassicæ* containing young larva of *Aphelinus* devouring an aphid embryo. PAR is the larva. (× 85).
Fig. 34. Longitudinal section of a similar aphid containing full-grown larva of *Aphelinus* (PAR), which has devoured nearly all of the aphid's organs. (× 42).
Fig. 35. Egg of *Xystus brassicæ* in unhatched larva of *Diaeretus rapæ*. (× 200).
Fig. 36. Embryonic larva of *Xystus* (HYP) developing in half-grown larva of *Diaeretus*, the latter in body of an *Aphis pseudobrassicæ*. From serial sections. (× 75).
Fig. 37. Half-grown larva of *Xystus* (HYP) in abdomen of full-grown larva of *Diaeretus* (PAR), the latter in aphid. From serial sections. (× 75).
Fig. 38. Mature larva of *Xystus*. Dissected from parasitized *Aphis pseudobrassicæ*. (× 40).
Fig. 39. Pupa of *Xystus*. (× 40).
Fig. 40. Adult female *Xystus brassicæ* Ashm. (× 25).



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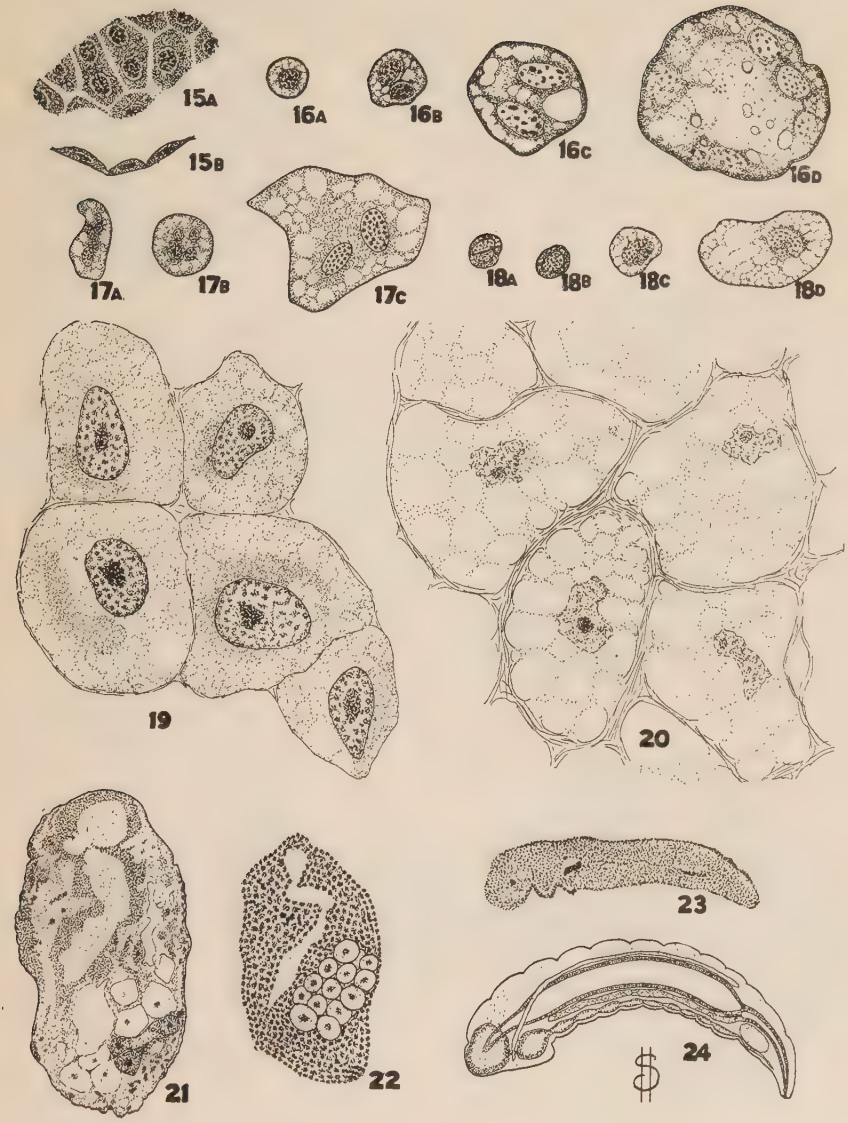

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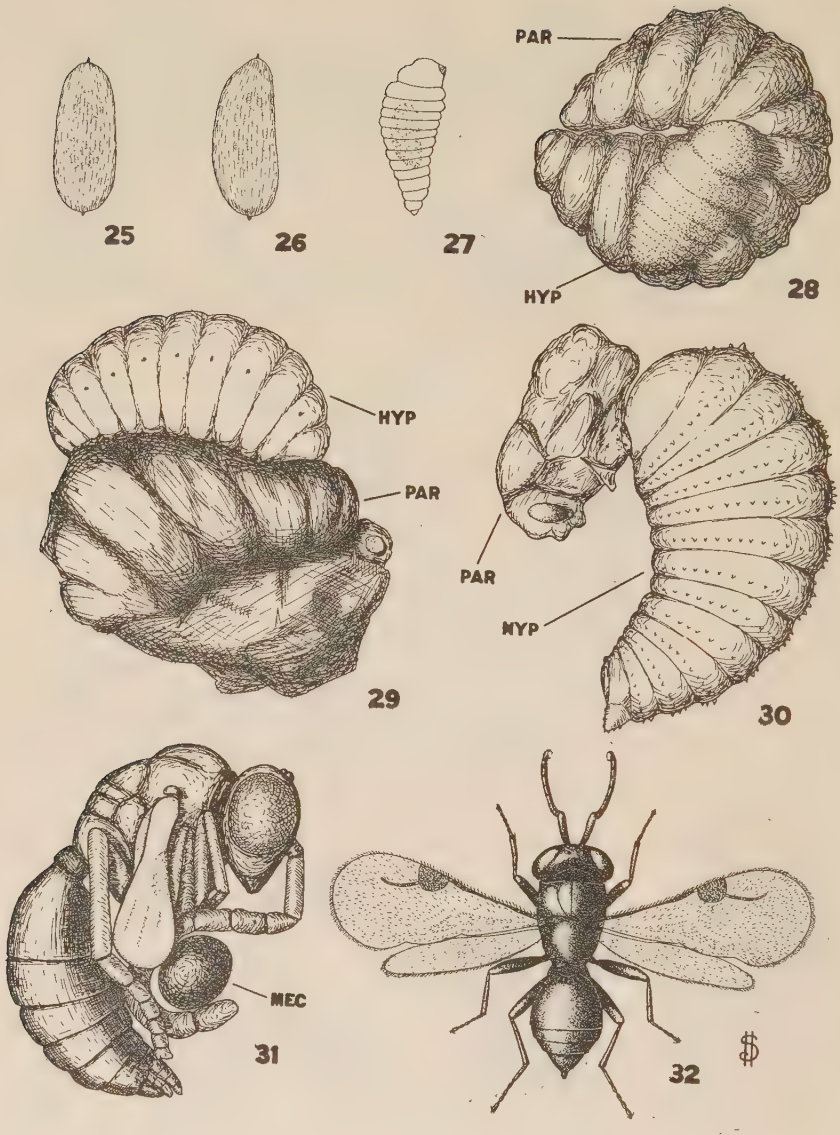

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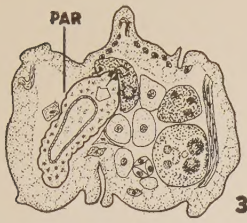

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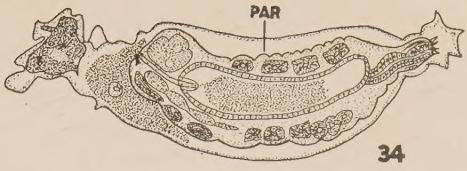

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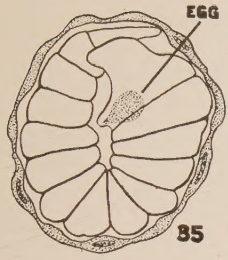

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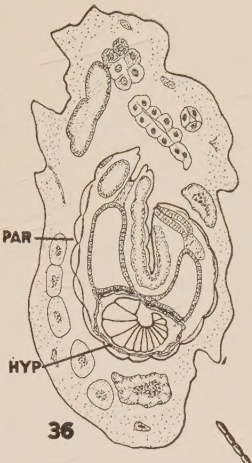

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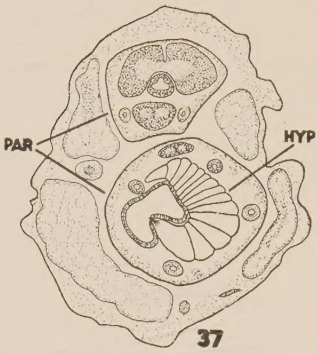

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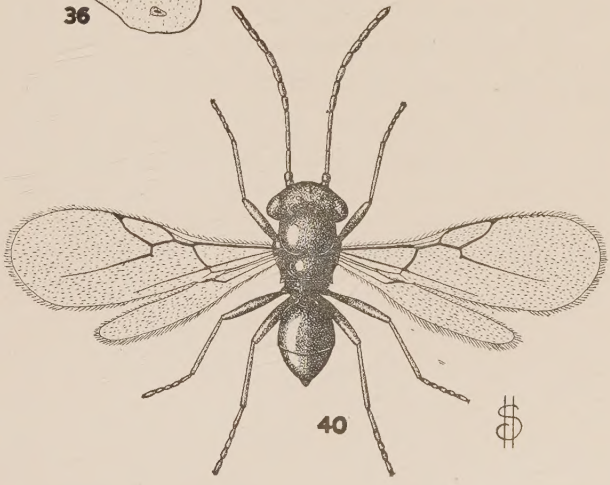

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Herbert Spencer

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I, Herbert Spencer, was born April 7, 1894, in Washington, D. C. A graduate of Hampton High School, Hampton, Va.; entered N. C. State College of Agriculture and Engineering September, 1911, and received the degrees of B. S. in Agriculture (Horticulture), 1915, and M. S. in Agriculture (Biology), 1917; was employed as Instructor in Zoology and Entomology at the N. C. State College 1915–1918, 1919–1920, as Assistant Professor of Entomology and Zoology, 1920–1921; enlisted as technician and officer-candidate for the Sanitary Corps, Medical Department, U. S. Army, 1918–1919; Graduate Student at the Ohio State University, 1917, 1921–1922, and Graduate Assistant in Entomology and Zoology, 1921–1922, receiving the degree of Doctor of Philosophy in Entomology, June, 1922.